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# The Correlation Between Testosterone and Spermatogenic Cell on Male Wistar Rats*(Ratus Norwegicus)* After The Treatment of Active Compunds of *Pluchea Indica*

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ABSTRACT

**Background:** *Pluchea indica*is applicable for anti-fertility on male wistar rats. It is then necessary to conduct further study on the correlation between testosterone and spermatogenic cell after the treatment of active compounds of *Pluchea indica* in various dosages. **Objective:** this study used experimental design by experimenting on 21 male wistar rats to under go 7 treatments and 3 repetitions. Randomized group design was employed with controlled treatment, tannin 4.7 mg, tannin 9.4 mg, tannin 14.1 mg, combination (alkaloid+tannin+flavonoid) 4.7 mg, combination (alkaloid+tannin+flavonoid) 9.4 mg and combination (alkaloid+tannin+flavonoid 14.1 mg). **Result**: The correlation between testosterone and spermatogenic cell was analyzed by means of Pearson correlation and regression. The findings of this study showed that p = 0.016; r = 0.458; r square 0.209 and the regression equation Y=1707.429+247.367x. The data p < 0.05 showed that there is correlation between testosterone and spermatogenic cell on male wistar rats after the treatment active compounds of *Pluchea indica* (combination of alkaloid+tannin+flavonoid) in various dosages (4.1 mg; 9.4 mg and 14.1 mg). **Conclusion:** There is correlation between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats after the treatment of active compounds of *Pluchea indica*. The tendency is that the lower the level of testosterone hormone, the lower the number of spermatogenic cellon male wistar rats after the treatment of active compounds of *Pluchea indica*



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## INTRODUCTION

There are some active compoundsof plant, to name alkaloid, flavonoid, tannin, and phenol [1]. *Pluchea indica* contains some active compounds such as tannin, alkaloid, and flavonoid [2] based on the study conducted [3]. Alkaloid is a base compound containing one or more nitrogen atoms, generally in combination of cyclic system and having prominent physiology activity that enables to be used for a wide range of medications. Flavonoid is currently under the investigation as it is rich of pharmacology activities. Tannin is an active compound of plant with a phenol characteristic and tart taste [4; 5].

Preliminary study on the faction of active compounds of *Pluchea indica* resulted in the followings: 1) the fraction of active compounds of fresh *Pluchea indica:* alkaloid fraction 0.120%, flavonoid fraction 1.047%, and tannin fraction 0.630% [6]; 2) the fraction of active compounds of dried *Pluchea indica*: alkaloid fraction 1.207%, flavonoid fraction 7.831%, and tannin fraction 1.8885% [7]. The extraction result of the active compound fraction was then made into fraction powder of alkaloid, flavonoid, and tannin.The preliminary study resulted in the data on different levels of active compound fraction between fresh and dried *Pluchea indica*. The difference on the active compounds has contributed to the choice of dried *Pluchea indica*to be made into powder.

Tannin contained in *Curcuma domestica*c lots spermatozoa; Cucurbitasin alkaloid suppresses the secretion of reproduction hormone, which is testosterone hormone, resulting in the troubled spermatogenesis process. Flavonoid of Momordica *charantina*inhibits aromatase enzyme, enzyme that catalyzes the conversion of androgen into estrogen and increases testosterone hormone [8]. Fraction of *Momordica charantia* also

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influences the growth of spermatogenic cell on male wistar rats [9]. The active compound of dried *Pluchea indica* in the form of powder has never been investigated before. The animals used in this experiment were male wistar rats as they belong to mammal taxonomy. This study aimed at proving the correlation between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats

According [10], alkaloid steroid causes the damage on the release of FSH (*Follicle Stimulating Hormone*) secreted from anterior hipofisa. FSH is a mediator to bind androgen in spermatogenesis; the damage in FSH results in the inhibited spermatogenesis [11] and the decrease on the quality of spermatozoa and thus infertility. There are two stages in spermatogenesis, namely spermatositogenesis, a process of altering spermatid into spermatozoa triggered by FSH hormone. Spermiogenesis is a process of forming spermatid into spermatozoa triggered by LH (*Luteinizing hormone)*. Spermatogenesis process occurs in seminiferous tubule testis. Spermatogenesis process is also controlled by testosterone hormone resulting negative feedback in hypothalamus and hipofisa axis.

Spermatozoa are produced during spermatogenesis process that occurs in seminiferous tubule testis. Spermatogenesis process is controlled by testosterone hormone. The detection of testosterone level is one of anti-fertility parameters [12]. The high or low level of testosterone (below the normal level) within the blood results in negative feedback on hypothalamus and disturbs spermatogenesis process. The normal level of testosterone triggers hypothalamus to secrete *gonadotrophin releasing hormone*and then triggers anterior hipofisa to secrete FSH and LH. FSH and LH trigger testis to perform spermatogenesis process [13;14].The level of testosterone hormone influences the number of spermatogenic cell (spermatogonia, spermatosit, spermatid, and spermatozoa) resulted from spermatogenesis. The active compounds contained in *Pluchea indica* influence hormone secretion during spermatogenesis process. It is then expected to be utilized as anti-fertility medication. The animals used in this experiment were male wistar rats as they belong to mammal taxonomy.

*Methodology:*

The population of this study was male wistar rats (*Ratus norwegicus)* aged 2-3 months with the average weight of 150-175 g. The samples were 27 male wistar rats to investigate the number of spermatogenic cells and the level of testosterone.

This study employed randomized group design with factorial research design that consisted of 2 factors. Factor 1 was the type of active compound fraction of *Pluchea indica* in the form of powder, namely tannin fraction; the combined fraction of alkaloid+flavonoid+tannin andcontrol. Factor 2 was the dosage, namely 4.7mg; 9.4 mg, and 14.1 mg. As a result, there were 9 combinations of treatment. The treatment combination of alkaloid+flavonoid+tannin was based on these calculations: 1) dosage 4.7 mg needed alkaloid 0.52 mg; flavonoid 3.37 mg; tannin 0.81 mg; 2) dosage 9.4 mg needed alkaloid 1.04 mg; flavonoid 6.74 mg; tannin 1.62 mg; 3) dosage 14.1 mg needed alkaloid 1.56 mg; flavonoid 10.11 mg; tannin 2.43 mg. The treatment was conducted within 7 days; and wistar rats were to live 51 days without any treatment. On day 58, 27 male wistar rats were killed by being injected isoflurane and decapitated [15] right away. Their abdomens were dissected to take their testis organ and blood from heart aorta. The testis organ is used for histology preparat of testis. The blood is taken to investigate the level of testosterone.

The histology preparat of testis was made by employing Humason method. The observation was conducted on seminiferous tubule transversely cut from 10 slices of testis organ. The calculation was administered on 3 tubules; 4 data were taken from every tubule, the upper right, upper left, bottom right, and bottom left parts (14). The observed parameters included the number of spermatogenic cells, namely spermatogonia, primary spermatosit, secondary spermatosit, spermatid, and spermatozoa [16].

For the purpose of investigating the testosterone level, blood was taken with a spuit from heath aorta and then centrifuged. The serum of the centrifuged blood was taken [17] before determining the testosterone level by employing ELFA (Enzyme Linked Flourencent Assay) technique. ELFA is a detection technique by utilizing serology method based on specific reaction between antigen and antibody, is high in sensitivity and specificity, and uses enzyme as an indicator [16].

The basic principle of ELFA is the analysis on interaction between antigen and antibody which is passively absorbed on the solid phase surface by utilizing the conjugate of antibody or antigen labeled as enzyme. Enzyme reacts with substrate and produces color. The emerging color is cumulatively determined by reading on 450 nm fluorescence. Fluorescent intensity was inversely proportional to the concentration of testosterone in the samples. In the last stage of the analysis, the result was automatically calibrated in memory and then printed. The concentration of testosterone sample was inversely proportional to the fluorescent intensity produced by conjugate reaction: substrate. The higher the fluorescent intensity, the less concentration of testosterone sample was [18]. The analysis on the testosterone hormone level was conducted in SIMA Laboratory Malang.

The correlation between testosterone level and spermatogenic cell was analyzed by means of Pearson correlation and regression.

## RESULTS AND DISCUSSION

The calculation of spermatogenic cells on histology preparat resulted in the number of spermatogenic cells on the controlled group, accounting averagely 3361 cells. The treatment of tannin and the combination of treatment (alkaloid+flavonoid+tannin) showed that the higher the dosage, the less spermatogenic cells were produced.

The result of analysis on testosterone level after treatment showed that 1) testosterone level of male wistar rats in the controlled group was 1.19 ng/dl; 4.46 ng/dl; 3.85 ng/dl; 2) in the treatment of tannin, the higher the dosage, the higher the testosterone level was; 3) in the combination of treatment (alkaloid+flavonoid+tannin) with the dosage of 4.7 mg and 14.1 mg, the testosterone level increased, but with the dosage of 9.4 mg, the testosterone level decreased.The correlation between the number of spermatogenic cell and the testosterone level on male wistar rats after the treatment of active compounds of *Pluchea indica* is presented in Figure 1.

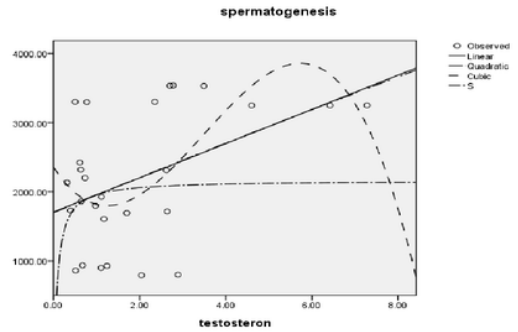


**Fig. 1:** The correlation between the number of spermatogenic cell and the testosterone level on male wistar rats

Based on the data presented in Figure 1, the number of spermatogenic cell and the testosterone level was then investigated by correlation and regression analysis to reveal the correlation between the two factors. The result of the analysis is presented in Table 1.

**Table 1:** The Summary of Correlation and Regression Analysis

| Correlation | | Regression Equation | Significance | r square | R |
|---|---|---|---|---|---|
| Testosterone | Spermatogenic cells | Y=1707.429+247.367x | p = 0,016 | 0,209 | 0,458 |

The analysis on the correlation between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats after the treatment of various types and dosages of active compounds of *Pluchea indica*resulted p = 0.016. The data showed p < 0.05 (Table 1); Ho "There is no correlation between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats after the treatment of active compounds of *Pluchea indica*(tannin and the combination of treatment:alkaloid+flavonoid+tannin) with various dosages(4.1 mg; 9.4 mg dan 14.1 mg)" was rejected. The hypothesis "There is correlation between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats after the treatment of active compounds of *Pluchea indica* (tannin and the combination of treatment:alkaloid+flavonoid+tannin) with various dosages (4.1 mg; 9.4 mg dan 14.1 mg)" was accepted.In other words, the finding showed that there is correlation between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats after the treatment of active compounds of *Pluchea indica* (tannin and the combination of treatment:alkaloid+flavonoid+tannin) with various dosages (4.1 mg; 9.4 mg dan 14.1 mg).

The regression analysis between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats after the treatment of various types and dosages of active compounds of *Pluchea indica* resulted regression equation Y=1707.429+247.367x with R square 0.209.The result of regression analysis showed that 20.9% spermatogenic cell was affected by testosterone and 79.1% was affected by other determining factors.

These findings are explained as follows: there is correlation and regression between testosterone and the number of spermatogenic cell. It is predicted that testosterone secreted by leydig cell has reached the targeted cell. Testosterone is secreted after the trigger of LH hormone into the targeted cell, which is leydig cell. The secretion of LH hormone triggers leydig cell to secrete testosterone. Testosterone plays its role in spermatogenesis process.

Hormone physiology within the blood is supported by a number of balance mechanisms between hormone producing gland and the targeted tissue. GnRH hormone secreted by hypothalamus spurs the synthesis and secretion of LH and FSH from anterior hipofisa; these hormones stimulate testis to synthesize and the release of

testosterone by leydig cell becomes the targeted organ. The high level of testosterone inhibits the system by reducing the synthesis and secretion of LH and FSH by anterior hipofisa.The decrease of LH results in the decrease of synthesis and testosterone by leydig cell. Normal testosterone level spurs the synthesis and secretion of GnRH by hypothalamus, then triggers LH and FSH from anterior hipofisa as well as simulates synthesis and secretion of testosterone by leydigcell [19;20]. LH, FSH and Testosterone function in spermatogenesis process on seminiferous tubule testis.

It is predicted that LH receptor in interstitial cell is not affected by the combination of active compounds of *Pluchea indica* with various dosages, but is affected by tannin, alkaloid, flavonoid so as to disturb LH in binding it self into the receptor; in this case LH stimulates the production of testosterone. The findings correspond to the fact that the treatment with active compound tannin and the combination of treatment (alkaloid+flavonoid+tannin) reduce the number of spermatogenic cells. This is in line with the statement [21] that active compounds of *Pluchea indica*constitute antispermatogenic on male wistar rats. According [22], testosterone and *androgen binding protein* affect epitel germinal tubule testis that interferes the production of spermatozoa. Testosterone and *androgen binding protein* or dehidrotestosterone maintain the chemical cement from epididimis. The decrease on testosterone level results in the decrease on the number of spermatogenic cells. LH receptor affected by active compound tannin of *Pluchea indica* inhibits the process of testosterone biosynthesis. This results in the disturbed function of testosterone hormone during spermatogenesis process [23].

Hipofisis produces FSH and LH or ICSH (*Interstitial Cell Stimulating Hormone*). Testis produces testosterone hormone metabolized from leydig cell. FSH stimulates sertoli cell to form Androgen *Binding Protein* (ABP) that functions as loading testosterone into seminiferous tubule and epididimis. This mechanism is necessary to reach the required testosterone level until spermatogenesis occurs. In addition, sertoli cell metabolizes inhibin, nonsteroid hormone with feedback mechanism on inhibiting the excessive production of FSH [10].

There are three hormones affecting spermatogenesis, namely FSH, LH, and testosterone. Testosterone functions in the maturation of spermatozoa and fertility. LH stimulates the development of leydig cell and secretion of testosterone/estrogen. The control over LH secretion from hipofisis is performed by negative feedback of testosterone. The treatment of testosterone reduces the release of LH and FSH, and affects as the direct stimuli on spermatogenesis [24].

Sertoli cells produce ABP and inhibin. These cells do not secrete androgen, but contain aromatase, typical enzyme for changing androgen into estrogen. ABP maintains the high and stable supply of androgen in tubules. Inhibin inhibits the secretion of FSH. FSH and androgen maintain the function of gametogenic testis. The maturation of spermatid into spermatozoa is affected by androgen functioning in sertoli cell. FSH works during the final stages of spermatid maturation; besides, FSH stimulates the formation of ABP [25].

Hormone level tends to decrease due to active compounds of *Pluchea indica* functioning as fitosteroid that disturbs the permeability of leydig cell membrane producing testosterone [10]. Based on the statement [26] that active compounds of fresh *Pluchea indica*in the form of liquid extract affect the testosterone level of male wistar rats and supported by the previous study on the stewed *Pluchea indica* that affects the testosterone level of male wistar rats [27], the disturbed permeability of leydig cell membrane contributes to the disturbed transfer of food substances as the energy source of testosterone biosynthesis.In this case, testosterone remains functioning as masculinity compound and affects the spermatogenesis in testis [17]. It is evident that testosterone plays it role in spermatogenesis by producing less spermatogenic cells after the treatment of active compounds of *Pluchea indica*within various dosages. Testosterone is steroid hormone that controls sexual behavior based on the increase of proper stimuli as well as changes the synthesis of enzyme, receptor, and protein that affect neurotransmitter function [28]. In addition, testosterone stimulates spermatogenesis process and lengthens the life span of spermatozoa in epididimis; the lower and the higher testosterone from the normal level, the more inhibited the spermatogenesis process is [29].

Spermatogenesis is the process of forming spermatozoa from spermatogonium, through complex and regular development stages. Spermatogenesis occurs in seminiferous tubule testis, under going several processes, namely proliferation, differentiation, and transformation. In seminiferous tubule testis, there are a number of cell groups with germinal cells that arrange several layers; every layer represents different generation. From Lamina basalis to lumen seminiferous tubule, the layers of spermatogonia, spermatosit, spermatid, and spermatozoa which are close to lumen will appear [30]. According [31], spermatogenesis is a series of processes that include proliferation, differentiation, and the maturation of spermatogenic cells; hindrance in one stage affects the development of spermatozoa. This is in accordance with the findings of study on histology testis preparat of male wistar rats that showed the layers of spermatogonium, spermatosit, spermatid,and spermatozoa close to lumen.

The spermatogenic cells found during spermatogenesis process include spermatogonia, primery spermatosit, secondary spermatosit, and spermatid. Spermatositogenesis is the development of reproduction cell

of spermatogonia into spermatid. Spermiogenesis is the development of reproduction cell from spermatid into spermatozoa. Spermatogenesis is triggered by FSH, LH, and testosterone hormones.

*Pluchea indica* contains active compounds: tannin, alkaloid, and flavonoid. Tannin, alkaloid, and flavonoid belong to fitosteroid group; the working mechanism of fitosteroid is predicted to resemble that of steroid hormone. Fitosteroid compound could be used as precursor of steroid sex hormone. This is as the result of similar structure between fitosteroid compound and cholesterol. Fitosteroid related to the receptor in nuclear membrane will reduce the activity of kinase protein that activates transcription and translation in protein synthesis [32]. Steroid hormone with receptor in nuclear membrane [29] is shown in Figure 2.

Tannin in the reproduction process disturbs the metabolism of spermatozoa. States [33] that some tanninis proven to inhibit enzymes during protein synthesis, clot protein, and form complex compound with high-energy phosphate resulting in the inactive phosphate in the body. Therefore, the metabolism energy as well as nutrition quality needed by spermatozoa will decrease, resulting in the disturbed spermatozoa life span.

Flavonoid in the reproduction system inhibits aromatase enzyme. States [33] that flavonoid inhibits kinase protein, and thus the inhibited formation of aromatase enzyme. The inhibited formation of testosterone into estrogen hormone results in the increase of testosterone hormone. Alkaloid in the reproduction system suppresses the secretion of reproduction hormone.

Fitosteroid binds the receptor in nuclear membrane and causes the alteration in receptor molecule. The altered receptor molecule changes the permeability of membrane. The receptor molecule altering the permeability of membrane affects the process of protein synthesis. The formation of protein synthesis in the cell contributes to the formation of intracellular protein that triggers the specific function of cell [32]. This protein is high in affinity for testosterone and dehidrotestosterone; if this process is inhibited, the proliferation of germinal cell is not possible to occur [34]. The inhibited secretion of *Androgen Binding Protein* and dehidrotestosterone results in the decrease of concentration in epididimis and the immaturities of spermatozoa.

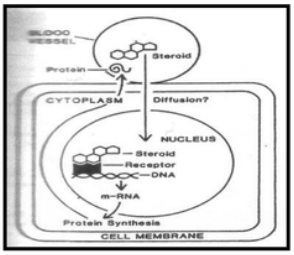


**Fig. 2:** Working Mechanism of Steroid Hormone (Hafez *et al*, 2008)

*Conclusion:*

There is correlation between the level of testosterone hormone and the number of spermatogenic cell on male wistar rats after the treatment of active compounds of *Pluchea indica*. The tendency is that the lower the level of testosterone hormone, the lower the number of spermatogenic cellon male wistar rats after the treatment of active compounds of *Pluchea indica*. The normal level of testosterone in blood will spur hypothalamus to produce GnRH; GnRH will spur hipofisa to secrete FSH and LH. FSH and LH spurs testis to synthesize testosterone in the spermatogenesis process. The lower and the higher testosterone from the normal level result in negative feedback to hypothalamus. Hypothalamus secretes little GnRH, and GnRH cannot spur anterior hipofisa to secrete FSH and LH.The level of FSH and LH in the blood decreases, unable to spur testis. Testis fails to synthesize testosterone and thus the disturbed spermatogenesis process.

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